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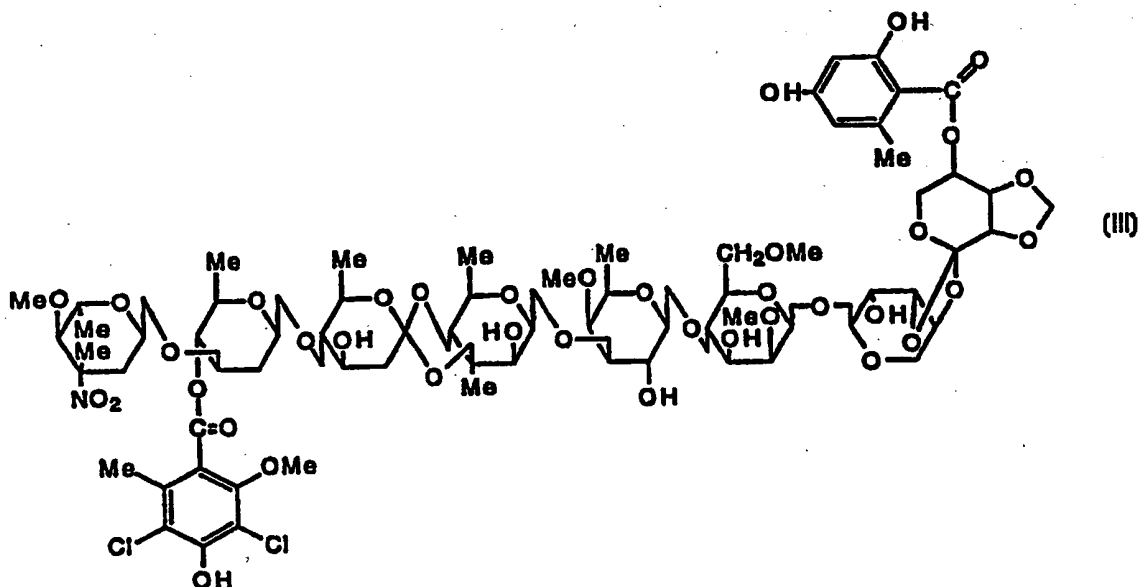
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(54) Title: COMPOSITIONS CONTAINING LIPOPHILIC OLIGOSACCHARIDE ANTIBIOTIC AND ALBUMIN



(57) Abstract

An aqueous pharmaceutical composition comprising a lipophilic oligosaccharide antibiotic salt, e.g., the N-methylglucamine salt of the everminomicin-type antibiotic of Formula (III) together with a binding agent such as human serum albumin or recombinant human albumin and a tonicity agent such as mannitol, is disclosed.

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Various strains of bacteria such as gram-positive cocci, e.g., streptococci and enterococci as well as methicillin-resistant and methicillin-susceptible staphylococci have become, and continue to become resistant to commercially available antibiotics, e.g., vancomycin. Such sensitive and resistant strains of gram-positive bacteria are an important cause of hospital-acquired and community-acquired infections. Such bacteria are recognized as significant pathogens that cause life-threatening illnesses. The commercially available antibiotics such as methicillin, macrolides, penicillins, quinolones as well as vancomycin have limitations including the aforesaid resistance and sensitivity to gram-positive bacteria.

Thus, there is a need for pharmaceutically acceptable compositions for treating bacterial infections including methicillin-resistant and methicillin-susceptible staphylococci and vancomycin-resistant bacteria. There is also a need for improved pharmaceutically acceptable compositions containing a lipophilic oligosaccharide antibiotic active against a broad range of susceptible gram-positive and gram-negative bacterial infections, especially pharmaceutical compositions adapted for parenteral use which avoid occurrence of the adverse reaction syndrome.

BRIEF SUMMARY OF THE INVENTION

Surprisingly, we have discovered a means by which lipophilic oligosaccharide antibiotics having good antibacterial activity against susceptible gram-positive and/or gram-negative bacterial infections may be delivered to animals, especially mammals such as man, afflicted with susceptible gram-positive or gram-negative bacterial infections, to provide effective treatment and/or prevention thereof while simultaneously avoiding occurrence of the adverse reaction syndrome. This means comprises combining a lipophilic oligosaccharide antibiotic with at least about a stoichiometric amount of a specified base and an amount of a binding agent such as natural human serum albumin ("HSA") or recombinant human albumin ("rHA") sufficient to achieve efficacious delivery of the lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding adverse reaction syndrome.

The present invention provides a composition of matter comprising:

- (a) a lipophilic oligosaccharide antibiotic represented by

Formula I;

5 COMPOSITIONS CONTAINING LIPOPHILIC OLIGOSACCHARIDE ANTIBIOTIC AND ALBUMIN

BACKGROUND OF THE INVENTION

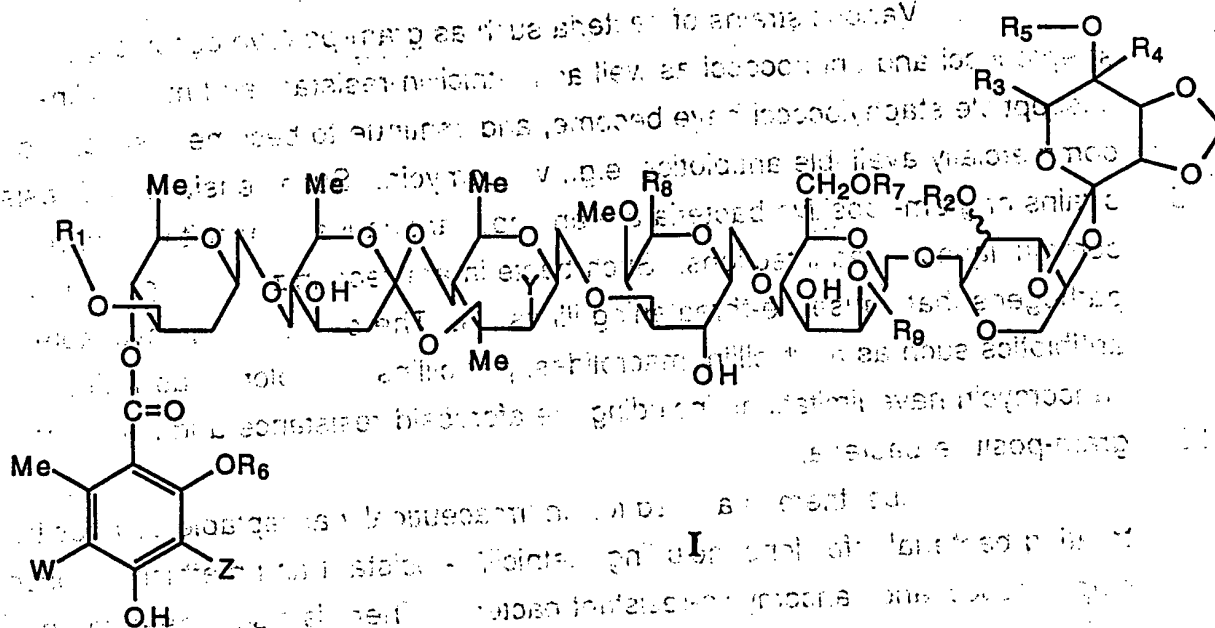
This invention relates to novel compositions of matter comprising a lipophilic oligosaccharide antibiotic salt together with a binding agent and to
10 pharmaceutical formulations containing such compositions of matter and to methods of making and using such pharmaceutical compositions to treat and/or prevent microbial infections in animals especially mammals such as human beings.

Lipophilic oligosaccharide antibiotics including, for example, everminomicins, curamycins, avilamycins and flambamycins are members of the
15 orthosomycin family of antibiotics which contain at least one acidic phenolic hydrogen, and two orthoester linkages associated with carbohydrate residues. See for example, A.K. Ganguly in "Kirk-Othmer, Encyclopedia of Chemical Technology", (1978), Volume 2, pp. 205-209, Third Edition, John Wiley and Sons and W.D. Ollis, et al., Tetrahedron (1979), Volume 35, pp. 105-127. These lipophilic
20 oligosaccharide antibiotics exhibit broad spectrum biological activity against gram positive and some gram negative bacteria in various in vitro assays, and in vivo activity in animal models such as mice. It has been observed that injection of these lipophilic oligosaccharide antibiotics cause an adverse reaction syndrome. The term "adverse reaction syndrome" as used herein means symptoms of the following type
25 observed in animals such as mice upon parenteral administration of lipophilic oligosaccharide antibiotics: incoordination, ataxia, lateral recumbency, urination, hind leg rigidity, labored breathing, and arrest.

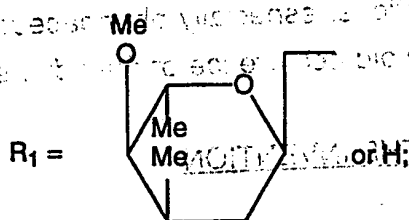
International Publication No. WO 93/07904 discloses pharmaceutically acceptable compositions containing an amount of a pharmaceutically acceptable
30 non-ionic surfactant and lipophilic oligosaccharide antibiotics, e.g., the everminomicin antibiotic of formula III with at least a stoichiometric amount of a base and an amount of hydroxypropyl- α -, β - or γ -cyclodextrins sufficient to achieve efficacious delivery of the oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding adverse reaction syndrome.

35 Cyclodextrins provide efficacious delivery of the oligosaccharides antibiotic to the serum but cyclodextrins are expensive materials and not a GRAS (generally regarded as safe) material. There is a need for alternative pharmaceutically acceptable compositions of lipophilic oligosaccharide antibiotic salts which are solubilized by agents other than cyclodextrins.

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wherein



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X is one of NO_2 , NO , NH_2 , NHCOCH_3 , NHOH , $\text{NH}(\text{C}_2\text{H}_5)$, $\text{N}(\text{C}_2\text{H}_5)_2$, OH or H ;

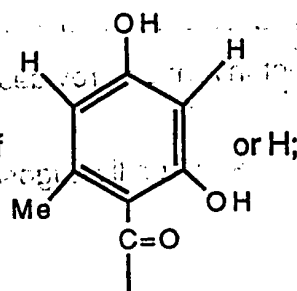
R_2 is one of CH_3 , $\text{COCH}(\text{CH}_3)_2$, COCH_3 , $\text{CO}(\text{CH}_2)_3\text{CH}_3$, COCH_2CH_3 or H ;

10

R_3 is one of CH_3 or H ;

R_4 is one of COCH_3 , $\text{CH}(\text{OCH}_3)(\text{CH}_3)$, $\text{CH}(\text{OH})\text{CH}_3$, CHO , or H ;

R_5 is one of

or H ;

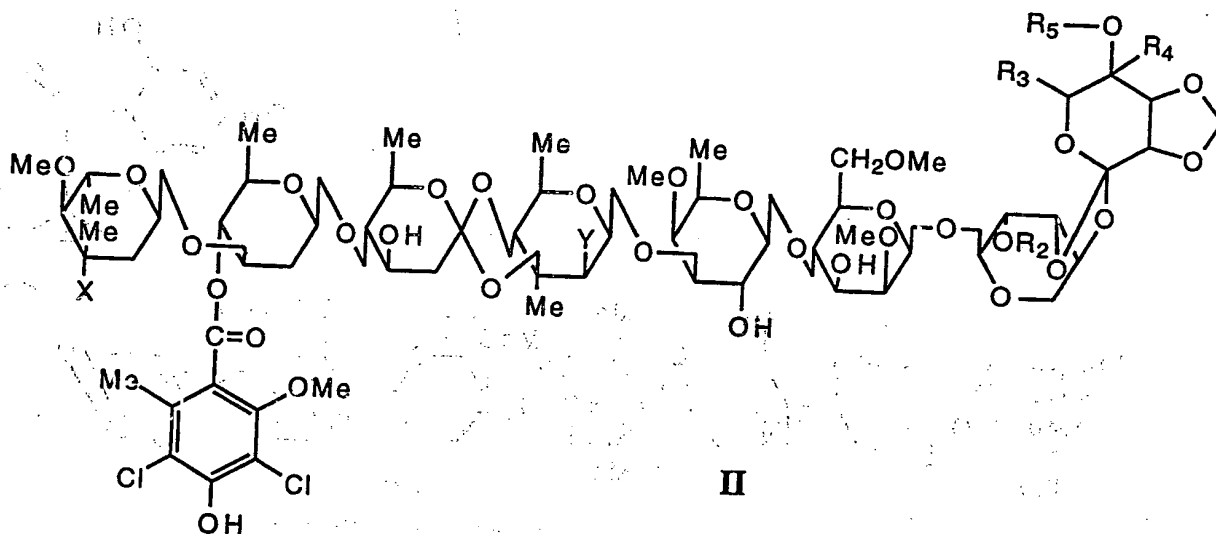
R_6 is CH_3 or H ;
 R_7 is CH_3 or H ;
 R_8 is CH_3 , CH_2OH or H
 R_9 is CH_3 or H ;

Y is OH , CH_3 , or H ;
 W is Cl or H ; and
 Z is Cl or H .

- (b) at least about a stoichiometric amount of a base capable of forming a pharmaceutically acceptable salt with a lipophilic oligosaccharide antibiotic of Formula I;
 (c) an amount of a binding agent sufficient to achieve efficacious delivery of said lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding adverse reaction syndrome; and
 (d) 0 percent by weight (basis, the total weight of said composition) up to an iso-osmotic amount of a pharmaceutically acceptable tonicity agent.

The present invention also provides a composition of matter comprising

- (a) a compound represented by the Formula II

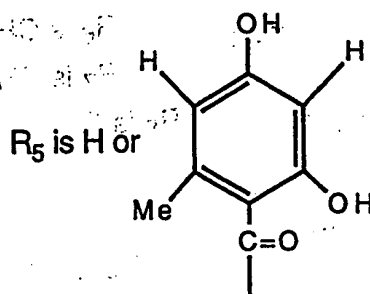


wherein X is one of NO_2 , NO , NHOH , NH_2 , NHCOCH_3 , NHC_2H_5 , $\text{N}(\text{C}_2\text{H}_5)_2$, OH or H
 Y is OH or H

R_2 is H or CH_3

R_3 is H

R_4 is H or $\text{CH}(\text{OCH}_3)(\text{CH}_3)$
 and



(b) at least about a stoichiometric amount of a base capable of forming a pharmaceutically acceptable salt with a lipophilic oligosaccharide antibiotic of Formula II;

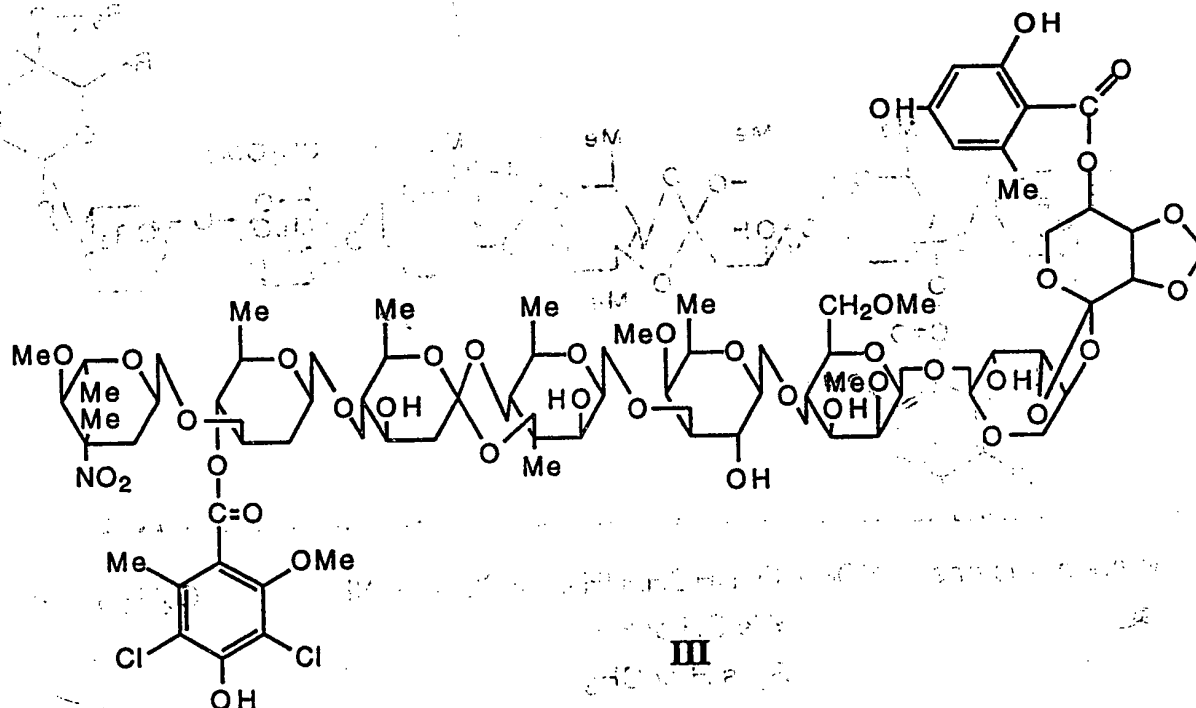
(c) an amount of a binding agent sufficient to achieve efficacious delivery of said lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding occurrence of adverse reaction syndrome; and

(d) 0 percent by weight (basis, total weight of said composition) up to an iso-osmotic amount of a pharmaceutically acceptable tonicity agent.

The present invention further provides a composition of matter

comprising

(a) the antibiotic compound represented by Formula III



(b) at least about two equivalents of a base (per mole of the compound of Formula III) capable of forming a pharmaceutically acceptable salt of the compound

delivery of said lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding occurrence of adverse reaction syndrome; and (d) 0 percent by weight (basis, said antibiotic of Formula III) up to an iso-osmotic amount of a pharmaceutically acceptable tonicity agent.

5 A preferred composition of matter of this invention contains the following: (a) the compound represented by Formula III, (b) N-methylglucamine, (c) recombinant human albumin ("rHA"), and (d) an iso-osmotic amount of mannitol; wherein the molar ratio of (a):(b):(c) is 1: about 3-3.5: about 0.018-0.030 and the amount of the mannitol the preferred tonicity agent, is about 35 to 45% weight
10 percent (basis total composition).

Pharmaceutical compositions formed by admixing a composition of matter comprising a compound represented by Formulas I, II or III and at least about a stoichiometric amount of a base capable of forming a pharmaceutically acceptable salt thereof and an amount of a binding agent with a pharmaceutically acceptable
15 tonicity agent and pharmaceutically acceptable carriers as well as methods of using such pharmaceutical compositions for treating or preventing susceptible gram positive and gram negative bacterial infections in animals, especially mammals in need of such treating or preventing are also provided.

As a preferred form of the invention, the aforesaid pharmaceutical
20 compositions are particularly applicable to parenteral administration, especially in vivo administration to human beings by the intravenous (IV) route.

The present invention also provides a method of preventing adverse reaction syndrome in animals following parenteral injection of a lipophilic oligosaccharide antibiotic represented by Formula I, II or III while simultaneously
25 delivering an antiinfective amount of said antibiotic to an animal, which method comprises parenterally administering to said animal an amount of a composition of matter of this invention sufficient for such purpose together with a pharmaceutically acceptable carrier.

30 BRIEF DESCRIPTION OF THE FIGURES

Figure 1 graphically illustrates the progression, with time, of a typical fermentation of Micromonospora carbonacea, var. africana, NRRL 15099, ATCC
39149.

35 DETAILED DESCRIPTION OF THE INVENTION AND OF THE PREFERRED EMBODIMENTS

Lipophilic oligosaccharide antibiotics, for example everninomicin antibiotics, exhibit useful in vitro antibacterial activity but do not readily form complete aqueous solutions suitable for safe and effective in vivo administration (i.e.

without occurrence of adverse reaction syndrome). Moreover, salts of these antibiotics formed by admixing at least about a stoichiometric amount of a base useful in this invention, e.g. the base, N-methylglucamine ("NMG") do not form complete aqueous solutions, at useful pH values. When said salts were added to water at useful concentrations of salt, we observed that only colloidal dispersions were formed. These colloidal dispersions tended to aggregate, and eventually gelled, especially in the presence of absorbed carbon dioxide and when the pH of such colloidal dispersions was less than about 9.3. We observed that complete aqueous solutions were formed by increasing the molar ratio of NMG to the compound of Formula III from 2:1 or 3:1 up to 12:1, but that the solution so formed with the 12:1 molar ratio had an undesirably high pH, was highly buffered and was irritating. Thus, we observed that parenteral injection into rats of higher primates, such as monkeys, of an aqueous formulation containing 12 moles of NMG per one mole of the compound of Formula III did not result in adverse reaction syndrome (presumably caused by gelling and precipitation of the compound of Formula III). The formulation, produced irritation upon injection, which irritation is presumably caused by the large amount of NMG base and resulting high pH at the injection site. However, parenteral administration of a composition containing 3 or 5 moles of NMG per mole of the compound of Formula III gave rise to adverse reaction syndrome. Surprisingly, we have found that a composition of matter comprising a lipophilic oligosaccharide antibiotic compound of Formula III, a specified amount of a specific base, e.g., NMG and a specified binding agent, e.g., human serum albumin in specified amounts, provides, when admixed with pharmaceutically acceptable carriers, especially sterile water for injection, a formulation which may be used safely and effectively for in vivo administration. Surprisingly, we have found that when 1 mole of a lipophilic oligosaccharide antibiotic, e.g., the compound of Formula III was admixed with 3 moles of a suitable base, e.g., NMG, in water and with 0.027 moles of human serum albumin, a clear aqueous solution of the complex was formed and the parenteral injection of such complexes into mice did not cause adverse reaction syndrome even at high doses, i.e., 400 mg of such complex per kg of body weight. See Table 1.

As will be evident from the in vivo results summarized in Table 1 parenteral injections of the aqueous dispersions of salts, e.g., the NMG salt of the eveminomicin-type antibiotic of Formula III into mice and rats gave rise to the adverse reaction syndrome. When the aqueous solutions of one of the compositions of matter of this invention such as one containing a specified binding agent, e.g., human serum albumin with the NMG eveminomicin-type antibiotic of Formula III salts were injected into the animals the occurrence of adverse reaction syndrome was wholly avoided.

Table 2 shows that adverse reaction syndrome can be reduced or completely avoided by parenteral injection into mice of clear aqueous solutions of NMG salts of the everninomicin-type antibiotic of Formula III with the specified binding agent, human serum albumin, of this invention.

- 5 Table 3 illustrates that increasing the molar ratio of base to the everninomicin-type antibiotics of Formula III from 2:1 to 9:1 wholly eliminates the occurrence of adverse reaction syndrome at all concentrations tested upon parenteral injection into mice.

COMPARATIVE EXAMPLE 3TABLE I

THE OCCURRENCE OF ADVERSE REACTION SYNDROME AFTER
ADMINISTRATION OF AQUEOUS FORMULATIONS OF ONE MOLE OF THE
COMPOUND OF FORMULA III: AND OF
3 MOLES NMG WITH AND WITHOUT HSA¹

10	Mice	% Adverse Reaction Syndrome ² at the following DOSES (MPK ³)					
		<u>160³</u>	<u>200³</u>	<u>320³</u>	<u>400³</u>	<u>520³</u>	
	III ⁴ : 3NMG	100	100	--	--	--	
	III: 3NMG: 0.027HSA ⁵	--	0	0	0	40	
15	Rats	% Adverse Reaction Syndrome at the following DOSES (MPK ⁶)					
		<u>60⁶</u>	<u>80⁶</u>	<u>100⁶</u>	<u>160⁶</u>	<u>200⁶</u>	<u>300⁶</u>
	III: 3NMG	60	100	100	100	100	--
	III: 3NMG: 0.027HSA ⁵	--	--	0	0	0	40
20							

Footnotes to Table 1

1. Human serum albumin
2. Adverse Reaction Syndrome symptoms were observed in the animals within 2 minutes after IV injection. (single dose)
3. MPK is mg of drug per kg of body weight of the mice (groups of 10, CF1, average weight 20g, Harlan Sprague - Dawley fasted 18 hours.)
4. III is the everminomicin-type antibiotic compound represented by Formula III.
5. 0.027 moles HSA is equivalent to 9% w/v HSA based on an 80mg/ml solution of the compound of Formula III.
6. MPK is mg of drug per kg of body weight of the rats (groups of 5, average weight 180-200g, Charles River, fasted 18 hours)

Table 3

Effect of Concentration of Lipophilic Oligosaccharide Antibiotic NMG Salt and Molar ratio of NMG to Antibiotic upon Adverse Reaction Syndrome¹ in Mice

5

<u>Drug of Formula III</u> (Drug Concentration)	<u>% ARS¹ at the following doses (MPK²):</u>				
	<u>50</u>	<u>100</u>	<u>200</u>	<u>300</u>	<u>400</u>
III:2NMG (10 mg/ml)	0	10	50	--	--
III:2NMG (20 mg/ml)	0	20	100	100	--
III:2NMG (50 mg/ml)	70	100	--	--	--
III:3NMG (20 mg/ml)	--	0	0	0	20
III:3NMG (40 mg/ml)	--	0	0	15	40
III:3NMG (80 mg/ml)	--	--	100	--	--
III:5NMG (20 mg/ml)	--	0	0	0	--
III:5NMG (50 mg/ml)	--	--	0	--	40
III:9NMG (20 mg/ml)	--	0	0	0	0
III:9NMG (40 mg/ml)	--	0	0	0	0
III:9NMG (80 mg/ml)	--	0	--	--	0

10 Footnotes to Table 3

¹ Adverse Reaction Syndrome Symptoms observed in mice (groups of 5 to 10, CF-1, average weight 20g, Harlan Sprague Dawley, fasted 18 hours) within 2 minutes after IV (single dose) injection

² MPK is mg of the drug of Formula III per kg of body weight.

15 ³ III is everminomicin-type antibiotic compound represented by Formula III.

TABLE 4

**PD 50 Values Showing Efficacy of Composition Containing the
Compound of Formula III Against Bacterial Infections¹ in CF1 Mice²**

DRUGS⁴

5	Organism ³	No. x 5 LD10	CFU ⁶ Mouse	A	B	C	D
	S. aureus 85111205	10	6.3x10 ⁹	9	11	26	--
	S. aureus 78100502	20	2.2x10 ⁸	12	12	6	--
	E. faecalis 88032810	10	9.7x10 ⁸	21	16	10	115
	E. faecalis 91031103 Vancomycin resistant	1.7	1.5x10 ⁹	60	60	100	49

Footnotes for Table 4

- 10 1 Injections were by IP route
- 2 CFI mice are Charles River CFI, ca 20 g while male mice
- 3 a Staphylococcus aureus 85111203 and 78100502
- b Enterococcus faecalis 803280 (Perm. APH-(3)-III) and 91031103 (Vancomycin resistant)
- 15 4 PD₅₀ Dosing routes for drugs: IV for A, B and C; SC for D in mg/Kg of body weight
- Drug A is compound of formula III: NMG:HPBCD in the molar ratio of 1:3:5 with Polysorbate 80 and mannitol in sterile water for injection (SWFI)
- 20 Drug B is the compound of formula III: NMG:HSA in the molar ratio of 1:3:0.027 and mannitol in SWFI
- Drug C is Vancomycin HCl intravenous available from Eli Lilly Co., Indianapolis; conc 10 mg vancomycin in 1 ml of SWFI).
- 25 Drug D is Amikacin
5. Multiple of one LD₁₀₀ (lethal dose to kill 100% of animals) of organism given to mice. (Measure of severity of the infection.)
6. Amount of organism administered to mice in Colony Forming Units ("CFU's").
- 30

Perhaps even more surprisingly, we observed that the Minimum Inhibitory Concentrations ("MIC") in the *in vitro* models, and the 50% protective dose ("PD₅₀") values in an *in vivo* mouse protection model, of the composition of 0.027 human serum albumin in combination with one mole of the compound of Formula III and 3 moles of NMG (Drug B in Table 4) were essentially the same as the MICs and

PD₅₀ values for the compound of Formula III and for those of the NMG salt of the compound of Formula III with HP β CD in said model (Drug A in Table 4). See Table 4 for PD₅₀ values for the compositions of the compound of formula III including a preferred composition of the present invention (Drug B) compared to those for vancomycin (Drug C) and amikacin (drug D).

Thus, we have surprisingly discovered improved pharmaceutically acceptable compositions of matter containing a lipophilic oligosaccharide antibiotic salt and a binding agent such as HSA which allows effective delivery of such antibiotic to the serum of an animal such as a mammal especially a man afflicted with a bacterial infection susceptible to treatment by such lipophilic oligosaccharide antibiotics of Formulas I, II and III.

The term "binding agent" as used herein means a substance which has sufficient free highly lipophilic binding sites to achieve efficacious delivery of the lipophilic oligosaccharide antibiotics of formulas I, II and III to the serum of an animal while simultaneously avoiding occurrence of adverse reaction syndrome. Typically suitable binding agents include any protein with highly lipophilic binding sites as evidenced by its effective binding with fatty acids. Such proteins include natural human serum albumin ("HSA") and recombinant human albumin ("rHA"). HSA is produced as a product from fractions of collected blood. Human serum albumin is available from Armour Pharmaceutical Div., Rhone-Poulenc Rorer, 500 Arcola Rd., Collegeville, PA 19426 and Fluka Chemika-BioChemika, Industriestrasse 25, CH-9470 Buchs, Switzerland. rHA is prepared by use of recombinant techniques such as described in EP 0 683 233 published 22 November 1995 and is commercially available from Delta Biotechnology Ltd., Nottingham NG71FD, Great Britain. Other binding agents include deoxycholic acid and salts thereof, for example, sodium deoxycholate which is available from Sigma Chemicals Co., P.O. Box 14508, St. Louis, MO. 63178.

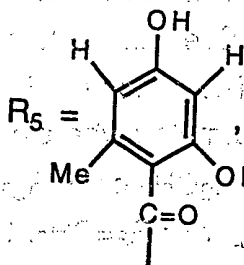
The moles of binding agent per mole of the compound of formula I, II or III varies from about 0.006 to about 0.03. If the binding agent is substantially free of impurities (i.e., contains <1-5%) such as fatty acids bound to the binding site of the agent, only about 0.006 moles (per mole of compound of formula I, II, or III) of such purified binding agent would be needed to achieve effective delivery of the compounds of formula I, II or III to the serum of the mammals. Amounts of binding agent above about 0.03 moles per mole of compound of formulas I, II or III have no further beneficial effect. The preferred binding agent is rHA which is readily available free of impurities such as fatty acids binding to it; about 0.006 moles of rHA (2% w/v based on 80 mg/ml of the compound of formula III) is needed to effectively transfer the compound of formula I, II or III to the serum of a mammal.

The term "tonicity agent", as used herein means an agent which allows the pharmaceutical compositions of the present invention to have an osmotic pressure compatible with human serum. Typically suitable tonicity agents, which may be present in the preferred pharmaceutical compositions of the present invention, include mannitol, sodium chloride, glycine and dextrose. The preferred tonicity agent (when one is used,) is mannitol but any pharmaceutically acceptable tonicity agent would also be acceptable.

The term "iso-osmotic" as used herein in reference to the amount of tonicity agent means the amount of the tonicity agent sufficient to make the pharmaceutical compositions of the present invention upon administration to a mammal iso-osmotic with the plasma of such a mammal. The iso-osmotic amount of tonicity agent varies with the tonicity agent used and may conveniently be measured in accordance with the procedures described in "Remington's Pharmaceutical Sciences" A.R. Gennaro, ed, 1990, 18th Edition, Mack Publishing Co., Easton, PA, Chapter 79 entitled "Tonicity, Osmoticity, Osmolality and Osmolarity", pages 1481-1498 at 1488-1491. The iso-osmotic amount of mannitol, the preferred tonicity agent, is preferably about 35 to 45% by weight basis total weight of all ingredients in the composition.

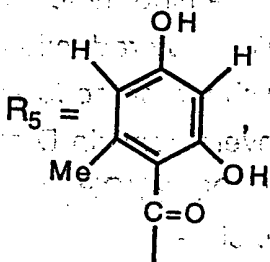
The bases found suitable for use in the present invention are those which form pharmaceutically acceptable salts of the lipophilic oligosaccharide antibiotics of Formulas I, II or III and include suitable organic and inorganic bases. Suitable organic bases include primary, secondary and tertiary alkyl amines, alkanolamines, aromatic amines, alkylaromatic amines and cyclic amines. Exemplary organic amines include the pharmaceutically acceptable bases selected from chlorprocaine, procaine, piperazine, glucamine, N-methylglucamine, N,N-dimethyl glucamine ethylenediamine, diethanolamine, diisopropylamine, diethylamine, N-benzyl-2-phenylethylamine, N-N'dibenzylethylenediamine, choline, clemizole, tris(hydroxymethyl)aminomethane, or D-glucosamine. The preferred organic bases include N-methyl glucamine ("NMG"), diethanolamine, and tris(hydroxymethyl) aminomethane ("TRIS"). Use of NMG in this invention is more preferred. The suitable inorganic bases include alkali metal hydroxides such as sodium hydroxide. The bases found useful in the preparation of compositions of matter of the present invention produce aqueous solutions having a pH of at least about 9.3. Lysine forms aqueous solutions having a pH of less than 9.3 and thus lysine is not a suitable base for the present invention. Divalent metal hydroxides such as the alkaline earth hydroxides, calcium hydroxide and barium hydroxide did not form aqueous solutions of the lipophilic oligosaccharide antibiotics of Formulas I, II or III in the presence of a binding agent having a pH of at least about 9.3 and were unacceptable as bases for use in the present invention.

The term "at least about a stoichiometric amount" as used herein in reference to the bases useful in this invention means the amount of base needed to substantially completely react with (i.e. result in more than 99% complete reaction) the acidic phenolic hydrogens of the lipophilic oligosaccharides antibiotics of Formulas I, II, III having one or two or three phenolic hydrogens. For the compounds of Formulas I and II wherein $R_5 = H$, there is only one phenolic acidic hydrogen per molecule and the stoichiometric amount of the pharmaceutically acceptable bases of this invention is at least about one mole of such base up to 12 moles of such bases. For the compounds represented by Formula I and II wherein



10

and for the compounds of Formula III there are three acidic phenolic hydrogens per mole of such compounds, the stoichiometric amount of base required to completely react with the three acidic phenolic hydrogens is at least one up to about 12 moles of the pharmaceutically acceptable bases useful in this invention. For the preferred lipophilic oligosaccharide antibiotics of Formulas I, and II wherein



15

and those of Formula III, it is preferred to use about one to 6 moles, and it is more preferred to use about 2.0 to 3.5 moles and most preferred to use about 2 to about 3 moles of a pharmaceutically acceptable base such as NMG to maintain the pH of an aqueous solution thereof at a value of about 9.3 as opposed to solutions having a higher pH, and which solutions were highly buffered when 6-12 moles of NMG were used.

20

The term "lipophilic oligosaccharide antibiotic" as used herein means selected lipophilic members of the orthosomycin family of antibiotics, more particularly flambamycin, the everminomicins, everminomicin-type antibiotics, curamycin and the avilamycin A-N antibiotics.

25

Flambamycin, a lipophilic oligosaccharide antibiotic produced by Streptomyces hygroscopicus DS 23230, whose structural Formula is that of Formula

I wherein $R_1=R_5=H$, $Y=OH$, $R_2=COCH(CH_3)_2$, $R_3=R_6=R_7=R_8=R_9=CH_3$, $R_4=COCH_3$ and $W=Z=Cl$ is disclosed by W.D.Ollis in Tetrahedron, (1979), **35**, 105-127.

Curamycin A is a flambamycin antibiotic (having a structural Formula represented by Formula I wherein $R_1, R_3, R_4, R_5, R_6, R_7, R_8, R_9, W$ and Z are the same as for flambamycin except $R_2=COCH_3$ and $Y=H$. See O.L. Galamarine et al. Tetrahedron (1961), **15**, 76 and V. Deulofer et al., Anales de Quimica (1972), **68**, 789.

Avilamycin A-N antibiotics are lipophilic oligosaccharide antibiotics isolated from an antibiotic complex produced by cultures of the organism

Streptomyces viridochromogenes, NRRL 2860. See J.L. Mertz et al. The Journal of Antibiotics (July 1986) Vol 39 (No. 7) 877-887. The structural Formulas for the avilamycin A-N antibiotics are represented by Formula I wherein, $R_1=R_5=H$, $Y=H$, $R_2=COCH(CH_3)_2$, $COCH_3$, $CO(CH_2)_3CH_3$, $COCH_2CH_3$ or H , $R_3=CH_3$, $R_4=COCH_3$, $CH(OH)CH_3$ or CHO and $R_6=CH_3$ or H ; $R_7=CH_3$ or H ; $R_8=CH_3$, CH_2OH or H ; $R_9=CH_3$ or H and $W=H$ or Cl and $Z=Cl$.

The everminomicin antibiotics useful in this invention include the everminomicins B, C and D isolated from the antibiotic complex produced by the organism, Micromonospora carbonacea var. carbonacea NRRL 2972 and a variety thereof M. carbonacea var. aurantiaca NRRL 2997 as described in USP 3,499,078.

The everminomicin derivatives having a nitrous, hydroxylation or amino moiety in place of the moiety in everminomicins B, C and D may be obtained by reduction of the nitro moiety in everminomicins B, C and D in accordance with the procedures of USP 4,006,225. A preferred everminomicin is N-acetylaminoeverminomicin-D and is represented by Formula II wherein $X=NHCOCH_3$, $Y=H$; $R_4=CH(OCH_3)(CH_3)$;

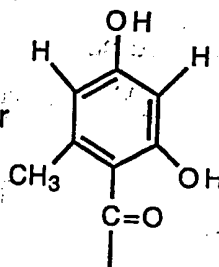
$R_3=R_5=H$ and $R_2=CH_3$. N-acetylaminoeverminomicin D and its di N-methylglucamine salt may be prepared by the procedures of USP 4,129,720 which discloses reduction of the nitro moiety of everminomicins B, C and D to produce the amino derivatives which are subsequently converted into the N-acyl e.g. N-acetyl, N-alkyl, e.g. $NH(C_2H_5)$, or N,N-dialkyl, e.g. $N(C_2H_5)_2$ derivatives. The preparation of the N-acyl-N-hydroxylamino everminomicin B, C and D derivatives and pharmaceutically acceptable salts thereof are also described. The preparation of Everminomicin 7 represented by Formula II wherein $X=OH$, $Y=H$,

$R_4=CH(OCH_3)(CH_3)$, $R_5=H$ and $R_2=CH_3$ is disclosed by A.K. Ganguly et al. in J. Chem. Soc., Chem. Comm., 1980, 56-58.

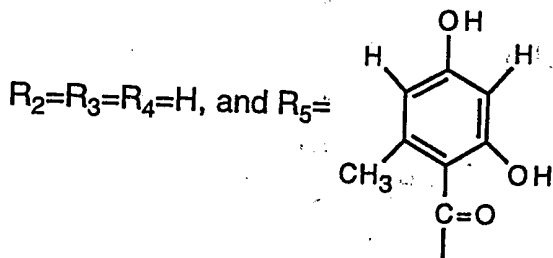
The "everminomicin-type" antibiotics are those lipophilic oligosaccharide antibiotics represented by Formula II wherein $X=NO_2$, NO , NH_2 , OH , $NHCOCH_3$, NHC_2H_5 , $N(C_2H_5)_2$, $NHOH$ or H , $Y=OH$, $R_2=CH_3$ or H ; $R_3=H$,

$R_4 = \text{CH}(\text{OCH}_3)(\text{CH}_3)$ or H and

$R_5 = \text{H}$ or



The compounds of Formula II wherein $X = \text{NO}_2$ or NH_2 , $Y = \text{OH}$



$R_2 = R_3 = R_4 = \text{H}$, and $R_5 =$

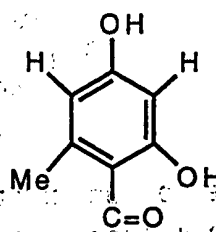
are isolated from an antibiotic 13-384

- 5 complex produced by fermentation of the organism Micromonospora carbonacea var. africana, NRRL 15099, ATCC 39149. Antibiotic components 1 (Formula II, $X = \text{NO}_2$ and Y , R_2 , R_3 , R_4 and R_5 are each defined as hereinabove in reference to antibiotic 13-384) and 5 (Formula II, $X = \text{NH}_2$ and Y , R_2 , R_3 , R_4 , and R_5 are as defined as hereinabove in reference to antibiotic 13-384) disclosed in USP 4,597,968 and
- 10 4,735,903 have the structural Formulas disclosed by AK Ganguly et al. in Heterocycles (1984) Vol. 28 (No. 1) p 83-88. The eveminomicin-type antibiotics of Formula II wherein $X = \text{H}$, NHOH , NHCOCH_3 and acyl and alkyl derivatives thereof are described in USP 4,622,314 and 4,767,748.

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The preferred compositions of matter of this invention include compounds of Formula II wherein $R_3=H$.

and	X	Y	R_4	R_5	R_2
	NO ₂	OH	CH(OCH ₃)(CH ₃)	H	CH ₃
	OH	H	"	"	"
	NO ₂	H	H	"	"
	NO ₂	H	CH(OCH ₃)(CH ₃)	"	"
	NHCOCH ₃	H	"	"	"
	NO ₂	OH	H	"	H



H
NHOH
NHCOCH₃
NH₂
NHC₂H₅
N(C₂H₅)₂

The most preferred everninomicin-type antibiotic is named 56-deacetyl-57-demethyl-45-O-de(2-methyl-1-oxopropyl)-12-O-(2,3,6-trideoxy-3-C-methyl-4-O-methyl-3-nitro- α -L-arabino-hexopyransoyl)-flambamycin 56-(2,4-dihydroxy-6-methylbenzoate) having the molecular Formula of: C₇₀H₉₇NO₃₈Cl₂ and the molecular weight of 1629 and is represented by Formula III.

The preferred compound of the Formula III may be obtained by fermentation of *Micromonospora carbonacea* var. *africana* NRRL 15099, ATCC 39149 or, more preferably, by an improved strain thereof, obtained as hereinafter described.

Utilizing the strain SCC 1413 of the culture NRRL 15099, ATCC 39149, the preferred compound of the Formula III may suitably be obtained by the procedures outlined in Example I of USP 4,597,968. In a specific example, in accordance with this procedure, the initial stage inoculum for the fermentation of strain SCC 1413 was prepared by transferring 2.5 ml of a frozen whole broth of 50 ml of the germination medium in 250 ml Erlenmeyer flasks. The germination medium consisted of beef extract, 0.3%; tryptone, 0.5%; dextrose, 0.1%; potato

starch, 2.4%; yeast extract, 0.5%; and calcium carbonate, 0.2%. The pH of the medium was adjusted to 7.5 prior to sterilization. The flasks were incubated at 30°C on a gyratory shaker at 300 rpm for 48 hours. For the second stage germination, 2 liter Erlenmeyer flasks containing 350 ml of the same medium were inoculated with a 5% volume of the first stage germination. The conditions for incubation were the same as before. A third inoculum stage was employed for all stirred tank fermentations and was prepared by a 24 hour incubation of the culture under the same conditions as employed for the second stage.

Ten liter fermentations were initially carried out in 14 liter NBS Laboratory Fermentors in a fermentation medium containing yeast extract, 0.5%; casein hydrolysate, 0.5%; cerelose, 1%; soluble starch, 2.0%; calcium carbonate, 0.4%; and cobalt chloride, 0.24 mg %. The pH of the medium was adjusted to 6.7 before sterilization and to 7.0 before inoculation. The third stage inoculum (2.5%) was used to initiate the fermentation which was conducted at 30°C with 0.35 vvm of air and 350 rpm agitation.

During the course of the fermentation, antibiotic production was monitored every 24 hours by bioassay of the whole broth against Staphylococcus aureus 209P (pH of the agar, 7.0) and Escherichia coli ATCC 10536 (pH of the agar, 8.0). The growth of the producing organism (packed cell volume), pH and dissolved oxygen levels were also determined either intermittantly or continuously. The course of a typical 10 liter tank fermentation is illustrated in Fig. 1.

We have developed an improved strain from SCC1413, NRRL 15099, ATCC 39149 using standard mutagenesis agents and obtained strains producing improved yields of the preferred everminomicin-type antibiotic compound of the Formula III. In a specific example, the parent strain SCC 1413, NRRL 15099, ATCC 39149 was exposed to an amount of the mutagenesis agent, N-nitrosoguanidine (NTG) sufficient to kill 90% of a culture of SCC 1413, ATCC 39149, NRRL 15099. Fifteen hundred surviving isolates were examined for enhanced biological activity against S. aureus and E. coli to determine which isolates exhibited improved production of the desired antibiotic of Formula III. The test procedure employed to determine enhanced activity was as follows: Single colony isolates were germinated in test tubes containing 10 ml of germination media of Example 1 of USP 4,597,968 and shaken at 250 rpm on a gyratory shaker at 30° C for 48 hours. Fermentation studies were initiated by transferring 2.5 ml of the seed to 250 ml Erlenmeyer flasks containing 50 ml of fermentation media and incubating at 30° C for 96 hours at 250 rpm on a gyratory shaker. The antibiotic obtained following fermentation was then assayed for improved antibiotic production by assessing the activity against S. aureus and E. coli and isolates giving improved yields of the

desired antibiotic were identified. The results for a representative improved isolate, designated herein as strain SCC 1631, are given in Table 5.

The foregoing strain-development procedure was repeated by subjecting the representative improved isolate, SCC 1631, to a further exposure to NTG, again in an amount sufficient to kill 90% of the cultures, followed by selection of the isolates on agar plates containing 150 $\mu\text{g/mL}$ of everninomicin D. Isolates giving enhanced production of the desired antibiotic were again selected by assessing biological activity thereof against *S. aureus* and *E. coli*. One such isolate, herein designated strain SCC 1756, was then utilized to produce the preferred antibiotic of Formula III.

Further, NTG mutagenesis of SCC 1756 yielded our current production strain, SCC 2146.

In the foregoing mutation procedures, the protocols for both studies were as previously described hereinabove. For the latter two mutation studies, fermentation broths were extracted with ethyl acetate and the concentrates were chromatographed on Whatman LKGF thin layer plates in a solvent system consisting of chloroform:methanol (9:1v/v) followed by bioautography against *S. aureus* and *E. coli* to confirm the production of all components of the antibiotic complex. To follow increased titers of the compound of Formula III, thin layer plates were examined by using the Shimadzu GS-930 TLC plate scanner and quantitating the higher producing extracts by using HPLC. Combined titers are defined as the sum of the compound of Formula III (antibiotic 13-384, component 1 of USP 4,597,968) and the nitroso analog of said component 1, i.e., antibiotic 13-384 component 1a.

Early observations indicated that although the parent strain SCC 1413 grew rapidly at 34°C, antibiotic production was optimal if the temperature was lower. This phenomenon was investigated as a means of fermentation optimization. Results of the temperature study indicated that optimal production was obtained when the temperature was lowered from 34°C to 30°C after 24 hours of incubation. All subsequent work in stirred tanks followed the protocol of incubating the fermentation at 34°C for 24 hours followed by lowering the temperature to 30°C for the duration of the fermentation run.

Media studies were conducted in conjunction with the isolation of the improved production strains. Carbon and nitrogen source substitutions were investigated as well as the addition of minerals and other complex nutrients. Replacement of casein hydrolysate by either meat or fish peptone and substituting potato dextrin (PDP 650) for soluble starch enhanced antibiotic production using strains SCC 1413 and SCC 1631. Subsequent enhancements in the production of the compound of Formula III were observed with the addition of corn steep liquor

and nickel (II) chloride in studies with strain SCC 1756. The current production fermentation media (4I + 1/2 Ni) optimized for the compound of Formula III contains glucose, 2.2 weight %; PDP 650 dextrin, 4.0 weight %; yeast extract, 0.5 weight %; meat peptone, 0.6 weight %; corn steep liquor, 0.5% vol., nickel chloride, 2.5×10^{-6} M; and calcium carbonate, 0.4 weight %. The pH of the medium was adjusted to 6.7 before the addition of calcium carbonate. Table 6 shows a comparison of the titers for strains SCC 1413, SCC 1631, SCC 1756 and SCC 2146 obtained in shake flask studies (50 ml of the current production medium in 250 ml erlenmeyer flasks, at 30° C, for 96 hours, at 300 rpm). The marked titer improvement (15 fold over the original parent, SCC 1413) is clearly demonstrated. Titters of 555-750 ug/ml (sum of the compound of Formula III and the nitroso derivative thereof) have been achieved in 100 liter fermentations using the current production medium with our best production strain, SCC 2146 (Table 7).

Strain		Titer (ug/ml)		Strain
Parent		Current		Parent
1413	100	100	100	1413
1631	100	100	100	1631
1756	100	100	100	1756
2146	100	100	100	2146

TABLE 5

Comparison of Strains SCC 1413 and SCC 1631 in FermentationsShowing Zones of Inhibition (mm) on Agar Plates¹TEST 1

<u>Strain</u> <u>SCC</u>	<u>S. aureus pH 7</u>		<u>E. coli pH 8</u>	
	<u>Undil.</u>	<u>1:20</u>	<u>Undil.</u>	
1631	28.7, 28.7	22.0	20,	17.5
1413	28.7, 28.7	19.0	12 H ³ ,	12H ³

TEST 2

<u>Strain</u> <u>SCC</u>	<u>S.aureus pH 7</u>		<u>E. coli pH 8</u>	
	<u>Undil.</u>	<u>1:20</u>	<u>Undil.</u>	
1631	28.1, 28.8	23.3, 23.1	14.8 C ² ,	15.0 C ²
1413	23.8, 23.1	20.5, 19.8	12 H ³ ,	12 H ³

1. Duplicate Determinations Where Appropriate

2. Clear Zone

3. Hazy Zone

Table 6

Flask Comparison of SCC's 1413, 1631, 1756 and 2146
Strains of Micromonospora Carbonacea var africana NRRL 15099, ATCC 39149
Titer of the compound of Formula III and Nitroso Analog (1A) Thereof (µg/ml)

<u>Culture</u>	<u>1 (NO₂)</u>	<u>1a (NO)</u>	<u>combined (1 + 1a)</u>
SCC 1413	5	3	8
SCC 1631	14	4	18
SCC 1756	17	16	33
SCC 2146	39	85	124

Table 7

100 Liter Fermentations of SCC 2146

Titer of Formula III and the NO Analog (1A) Thereof (µg/ml)

<u>Media</u>	<u>1 (NO₂)¹</u>	<u>1a (NO)²</u>	<u>combined (1 + 1a)</u>
4I	105	315	420
4I	135	170	305
4I + 1/2Ni ³	55	500	555
4I + Ni ⁴	150	575	725
4I + 1/2Ni ³	100	650	750
4I + 1/2Ni ³	130	470	600

15 Footnotes to Table 7

1. The everminomycin-type antibiotic of Formula III.
2. The Nitroso analog of the antibiotic of Formula III.
3. Nickel concentration (1/2 Ni) = $2.5 \times 10^{-6}M$.
4. Nickel concentration Ni = $5 \times 10^{-6}M$.

The isolation of the lipophilic oligosaccharide antibiotic complex containing the compound of Formula III and the nitroso analog thereof was accomplished by use of the procedures of Example 4G of USP 4,597,968. The fermentation broth was adjusted to pH 7 and extracted twice with a volume of ethyl acetate two times the volume of the fermentation broth. The combined ethyl acetate extracts were concentrated and the amounts of the compound of Formula III and the nitroso analog thereof were determined by HPLC. The nitroso analog was converted into the nitro compound of Formula III by use of an oxidizing agent such as tertiary butyl hydroperoxide (t-BuO₂H) with vanadyl acetylacetonate dissolved in an aprotic organic solvent at room temperature. The course of the reaction was monitored by, for example, HPLC. The reaction mixture was quenched with trialkylphosphite and the crude product was purified by standard chromatographic techniques. e.g. silica gel column chromatography (acetone/CH₂Cl₂) or a column containing a polyhydroxyvinyl polymer such as Fractogel (Toyo Pearl) available from Toyo Haas, Philadelphia, Pennsylvania.

The pharmaceutically acceptable composition of matter of this invention may contain, in addition to (a) an antibiotic of Formula I, II, III, (b) at least a stoichiometric amount of a base capable of forming a pharmaceutically acceptable salt of such antibiotics and (c) an a specified amount of a binding agent, preferably recombinant human albumin, and (d) 0 weight percent (basis total weight of the composition) to about an iso-osmotic amount of a pharmaceutically acceptable tonicity agent and pharmaceutically acceptable carriers. The preferred pharmaceutically acceptable carriers include sterile water for injection and others that produce pharmaceutically acceptable compositions, i.e., compositions which when dissolved in a pharmaceutically acceptable carrier are substantially free of visible particles.

BIOLOGICAL ACTIVITIES

We have surprisingly found that the preferred composition of matter of this invention, comprising one mole of the compound represented by Formula III, 3 moles of NMG and 0.027 moles of human serum albumin, has substantially the same geometric mean MICs (GMM) against various bacteria, and substantially the same serum protein binding values as the compound of Formula III per se. It is expected that all the compositions of matter of this invention will behave similarly.

The in vitro antibacterial activity tests were performed via conventional agar dilution methods in Mueller - Hinton agar. The GMMs for the above-listed preferred composition of matter of this invention and for the compound of Formula III were determined against various bacteria, e.g., gram positive and gram negative bacteria. The term "susceptible gram positive and gram negative bacterial

infections" means a broad range of gram-positive bacterial infections, e.g., methicillin-resistant and methicillin-susceptible staphylococci, various strains of streptococci and enterococci and some gram-negative bacterial infections, e.g., Moraxella, Haemophilus, and Legionella. The compound of Formula III had excellent activity (2-fold more potent than vancomycin) against both methicillin-resistant staphylococci (GMM, 0.1 µg/ml) and methicillin-susceptible staphylococci (GMM, 0.5 µg/ml). The compound of Formula III also had good activity (similar to that of vancomycin) against Enterococcus faecalis and Enterococcus faecium (GMM, 0.49 µg/ml) and similarly good activity against enterococci resistant to vancomycin (MICs, ≥ 128 µg/ml) and good activity (MICs, ≤ 0.5 µg/ml) against various strains of streptococci. The compound of Formula III was very active against Borrelia burgdorferi (MICs, ≤ 0.49 µg/ml) and Legionella pneumophila and L. longbeacheae (MICs 2.5 µg/ml) but was only slightly active against gram-negative bacteria (GMM, ≥ 760 µg/ml), Trichomonas vaginalis (MICs, ≥ 192 µg/ml) and Mycoplasma sp. (MICs 200 µg/ml). No cross resistance with other antibiotics was observed.

The compound of Formula III had moderate bactericidal activity against various clinical and laboratory strains of staphylococci. The bactericidal activity of the compound of Formula III against staphylococci and enterococci was similar to that of vancomycin. The compound of Formula III had good activity against staphylococci in mice (PD₅₀ range 0.5 to 25.0 mg/kg), similar to that of vancomycin (0.7 to 28.5 mg/kg).

Following IV administration (30 mg/kg) of the compound of Formula III and 2 molecules of NMG, high serum levels were seen in rats (peak about 90 µg/ml) with a long serum beta half-life.

The pharmaceutically acceptable compositions of matter of this invention are expected to be active against the above-listed susceptible bacteria as well as against spirochetes including Treponema pallidum, anaerobes including Clostridium difficile as well as against Pneumocystis, Toxoplasma, protozoa and helminths.

Based on the activity of the compound of Formula III against Borrelia burgdorferi, Legionella pneumophila and L. longbeacheae, we expect that the compositions of matter containing the compound of Formula III will exhibit activity in humans against Lyme disease and legionnaire's disease.

The present invention provides a method of treating or preventing susceptible gram-positive and gram-negative bacterial infections in animals by administering to such animals especially man afflicted with such infections an amount or a pharmaceutical composition of the compositions of matter of this invention and a pharmaceutically acceptable carrier therefor.

The compositions of matter of this invention may be combined with any pharmaceutically acceptable carrier, e.g., non-ionic surfactants, sterilized water, aqueous ethanol, vegetable oils, or polyols, e.g., polyethylene glycols and propylene glycol and administered orally, parenterally or topically in a variety of formulations.

5 The use of sterile water for injection as a carrier is preferred. The sterile water for injection may optionally contain pharmaceutically acceptable substances, e.g. sodium chloride, potassium nitrate, glucose, mannitol, dextrose, sorbitol, xylitol or buffers such as phosphate, acetate or citrate as well as preservatives.

10 The compositions of matter of this invention are prepared by admixing a lipophilic oligosaccharide antibiotic of Formula I, II or III with at least about a stoichiometric amount of a base capable of forming a pharmaceutically acceptable salt thereof in a suitable solvent such as water and with a specified amount of a binding agent, for example human serum albumin. The order of admixing is not critical, but preferably an aqueous solution of the specific binding agent is admixed
15 with the base or alternatively it may be added after the base is admixed with the lipophilic oligosaccharide antibiotic and a tonicity agent such as mannitol. The formation of the aqueous solutions may take place at a temperature between 2° and 25°C. The aqueous solution so formed is filtered to produce a clear aqueous solution of the complex which may be evaporated or preferably freeze dried to form
20 the compositions of matter of this invention in the form of a lyophilized powder which is readily reconstituted by addition of an amount of a pharmaceutically acceptable carrier such as sterile water for injection. The pharmaceutically acceptable non-ionic surfactant e.g. polysorbate-80, when used, would be added to the aqueous solution before filtration and lyophilization. Alternatively, the aqueous solution may be
25 frozen, thawed and thereafter filtered before use, e.g., as an intravenous IV formulation. It is a special feature of the present invention that the pharmaceutical compositions of the present invention form aqueous solutions and yet contain about 0.006 to about 0.03 mole, preferably about 0.027 mole of human serum albumin per mole of a compound of formula I, II or III. The discovery that pharmaceutical
30 compositions useful for safely and effectively delivering lipophilic oligosaccharide antibiotics to the serum of animals afflicted with susceptible bacterial infections, especially susceptible gram positive and gram negative bacterial infections, could be prepared by use of such a small amount of human serum albumin is particularly unexpected.

35 It is believed that even 0.006 moles of recombinant human serum albumin per mole of compound of formula I, II or III (substantially free of fatty acids and other impurities) will also be effective in the compositions of the present invention.

For oral administration, the compositions of this invention may be compounded in the form of tablets, capsules, elixers or the like. Tablets and capsules may contain such excipients as starch or lactose; liquid forms may contain coloring or flavoring agents. Topical preparations may be in the form of creams, hydrophobic and hydrophilic ointments, or aqueous, non-aqueous or emulsion-type lotions as well as pessaries or powders. Typical carriers for such formulations are water, oils, greases, polyesters and polyols. Parenteral formulations, e.g., injectable dosage forms, are usually liquids such as solutions or suspensions, with typical carriers being sterile water, saline solution and 5% by weight dextrose solutions. Parenteral formulations are preferred. Intravenous (IV) formulations are more preferred.

The dose to be administered in any particular dosage form will depend upon various factors, such as the weight, age and sex of the animal especially a mammal such as a human being being treated, the susceptibility of the infecting organism to the lipophilic oligosaccharide antibiotic, the stage and severity of the infection. Generally, the dosage of the lipophilic oligosaccharide antibiotics of Formula I, II or III administered is from about 1.0 mg to about 15 mg per kilogram of body weight, preferably about 3-5 mg per kilogram of body weight per day in divided dosages. The specified dosage is left to the discretion of the practitioner who will take into consideration the age and sex of the patient as well as, *inter alia*, the severity of the infection to be treated. IV administration is preferred.

In treating certain patients with the compositions of this invention, it is possible to include other pharmaceutically active ingredients in the same dosage unit.

EXAMPLES

EXAMPLE 1

A 100 liter fermentation of strain SCC 2146 of Micromonospora carbonacea var. africana NRRL 15099, ATCC 39149 improved as described hereinabove, was conducted in accordance with the procedures of Example 1B of USP 4,597,968 except that the following production medium (4I + 1/2Ni) was used and that the fermentation was conducted at 34°C for 24 hr followed by lowering the temperature to 30°C for the duration of the fermentation run, i.e., for another 72 hr (total fermentation time of 96 hr). Aeration and agitation rates were, 0.35 vvm and 350 rpm, respectively

Glucose	2.2% (weight)
PDP 650 dextrin	4.0% (weight)
Yeast Extract	0.5% (weight)
Meat Peptone	0.6% (weight)
Corn Steep Liquor	0.5% (volume)
Nickel Chloride	$2.5 \times 10^{-6} \text{M}$
Calcium Carbonate	0.4% (by weight)
Tap Water q.s. to give	1000ml

B. Isolation

Extract the fermentation broth of Example 1A twice with 200 L of ethyl acetate. Combine the ethyl acetate extracts and concentrate to provide a concentrated antibiotic complex containing a mixture of the compound of Formula III and the nitroso analog thereof (as determined by HPLC).

EXAMPLE 2

A) To 919g of antibiotic complex produced as described in Example 1 and containing 294g (32%) of a mixture of 3.4 moles of the nitroso analog to one mole of the compound of Formula III dissolved in 4.6 L of ethyl acetate, 68.8 g of NaHCO_3 and 2.98g of vanadyl acetylacetonate 3M in 2,2,4-trimethylpentane available from Aldrich (0.06 eq); 394 mL of 3M t-butylhydroperoxide was added to the so-formed mixture after a 1/2 hour period. Portions of 1.45g (0.03 eq) of vanadyl acetylacetonate were added thereto at 0 and after 1 1/2, 2 1/2, 3 1/2 and 4 hours so that 0.15 eq of vanadyl acetylacetonate was added over 4 hours. The reaction mixture was immersed in an ice bath, and 203 mL (0.5 eq) of triethylphosphite $(\text{C}_2\text{H}_5\text{O})_3\text{P}$ was added thereto. The so-formed reaction mixture was diluted with an equal amount of ethyl acetate while keeping the temperature of the reaction mixture at $\leq 30^\circ\text{C}$. The diluted ethyl acetate reaction mixture was washed twice with water. The aqueous layers were salted and extracted with ethyl acetate. The combined organic extracts were dried over MgSO_4 , filtered and concentrated. The so-formed residue was dissolved in a minimum amount of acetone and precipitated into 7 L of 1:9 (v/v) ethyl ether/hexane. The residue was filtered and washed with hexane dried under vacuum and heat to give 928g containing 30% (278g) of the nitro compound of Formula III.

B) The residue of Example 2A was purified on 5 kg of silica gel in a column. The column was eluted with 12 liters of CH_2Cl_2 containing successively 10%, 20%, 25%, 30%, 35% (v/v) of acetone. The appropriate fractions were combined and concentrated at $\leq 35^\circ\text{C}$. The so-formed residue was dissolved in acetone and precipitated into 10 parts of 10% ethyl ether/hexane. The product was

filtered and dried under vacuum without heat. The main fraction contained 147.5g of the compound of Formula III (98.7% pure). The other fractions contained crude product and were subjected to repeated silica gel chromatography until at least a 96-98 % pure product was obtained. The structure was determined by NMR and MS and found to be consistent with that of Formula III.

EXAMPLE 3

10 An aqueous solution containing 58.3g of manitol (USP and EP) and 42.0g of N-methyl glucamine ("NMG") USP and BP was prepared in 2.0L of water. To this solution was added 117g of the compound of Formula III followed by addition of 555g of 25% by weight HSA solution (Armour Pharmaceuticals). The so-formed solution was brought to a final volume of 2.9L. After agitation, a homogeneous solution containing 40mg per mL of the compound of Formula III was formed. The molar ratios of the three components were 1 mole of the compound of Formula III to 3 moles of "NMG" to 0.027 moles of HSA. The so-formed solution was filtered, filled into vials and freeze-dried to form a powder. For reconstitution of the powder to form a pharmaceutical composition, a pharmaceutically acceptable carrier such as sterile water for injection USP was added to the freeze-dried powder.

20 The safe and effective administration of the pharmaceutical composition of clear aqueous solutions of the homogeneous complex prepared in accordance with the procedures of this invention was tested in various in-vivo animal models by reconstituting the lyophilized powder with sterile water for injection to give solution containing 80 mg/mL of the compound of formula III such are reported in Tables 1 and 4. A similar procedure may be used to prepare pharmaceutical compositions containing recombinant human albumin ("rHA") by replacing HSA with an equivalent amount of rHA.

EXAMPLE 4

35 A 100-mL aqueous solution containing 160 mg/mL of compound of Formula III and 57.6 mg/mL of NMG was prepared as a stock solution. To each 10mL of this solution was added 1.6, 3.2, 4.8, 6.4 and 8.0 mL of 25% human serum albumin solution and sterile water for injection was added to the so-formed solution to provide a solutions with a final volume of 20 mL containing 80 mg/mL of the compound of Formula III. The molar ratio of the three components were 1 mole-of the compound of Formula III to 3 moles of NMG to 0.006, 0.012, 0.018, 0.024 and

0.03 moles of HSA. The solutions so formed were filtered and used for animal testing.

EXAMPLE 5

This example provides the preparation of the composition used for the studies in rats and mice summarized in Table 1. The procedure of Example 3 was followed using the following ingredients in the listed amounts:

Ingredients	mg/Vial	% W/W ¹
Compound of formula III	200	33.5
NMG US and BP	72	12.1
HSA USP and EP	225	37.7
Mannitol USP, BP and EP	100	16.7
Water for injection USP	sublimed	---

1) The weight of each ingredient to the total weight of all ingredients.

The molar ratio of III:NMG:HSA was 1:3:0.027

The reconstituted solution was not iso-osmotic but use of a total of 200 or 300 mg of mannitol (instead of only 100 mg as used above) would likely render the solution iso-osmotic.

EXAMPLE 6

This example illustrates preparation of a composition such as used in Table 2 and containing 2 to 10% W/V based of a solution containing 80 mg/mL of the compound of formula III. The procedure of Examples 4 and 5 were followed to prepare a solution containing the compound of formula III, NMG and HSA in the molar ratio of 1:3: 0.006 to 0.03.

Human serum albumin
(HSA)

Broad Range Studied

mg HSA per 200 mg of compound of formula III	50-250
moles per one mole of III	0.006-0.03
mg/mL per 80 mg/mL of III	20-100
% w/v/ based on 80 mg/mL of the solution containing III	2-10%

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EXAMPLE 7

This example provides compositions which may be prepared in accordance with the procedures of example 4 having a range of ingredients in compositions shown below.

10

Ingredient

Broad Range

Relative Molar Ratio

Mg/200 mg. of III

based on one mole of III

Compound of Formula III	200	1
NMG, USP and BP	48-144	2-6
HSA, USP and EP	50-250	0.006-0.03
Mannitol, USP, BP and EP	200-400	9.0-18
Sterile Water for injection USP	sublined	

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Ingredient

Preferred Range

Molar Ratio

20

Mg/200 mg. of III

based on one mole of III

Compound of Formula III	200	1
NMG, USP and BP	72-84	3-3.5
HSA, USP and EP	150-250	.018-.03
Mannitol, USP, BP and EP	200-300	9.0-13.5
Sterile Water for Injection, USP	sublim,ed	

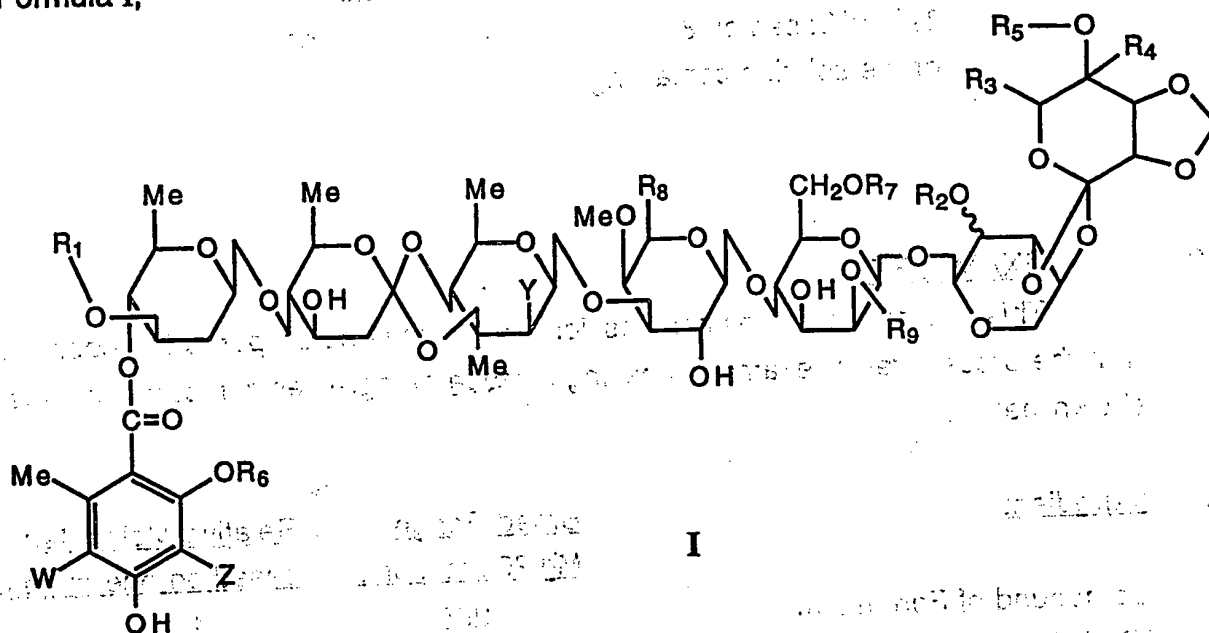
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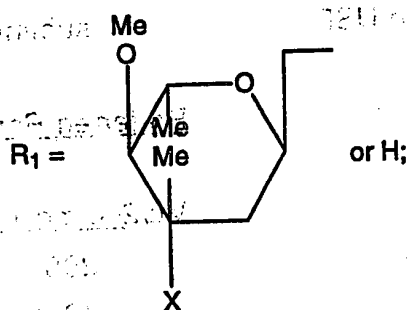
WHAT IS CLAIMED IS:

1. A composition of matter comprising:

(a) a lipophilic oligosaccharide antibiotic represented by Formula I;



wherein

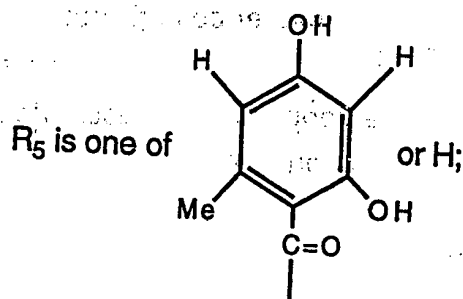


X is one of NO_2 , NO , NH_2 , NHCOCH_3 , NHOH , $\text{NH}(\text{C}_2\text{H}_5)$, $\text{N}(\text{C}_2\text{H}_5)_2$, OH or H ;

R_2 is one of CH_3 , $\text{COCH}(\text{CH}_3)_2$, COCH_3 , $\text{CO}(\text{CH}_2)_3\text{CH}_3$, COCH_2CH_3 or H ;

R_3 is one of CH_3 or H ;

R_4 is one of COCH_3 , $\text{CH}(\text{OCH}_3)(\text{CH}_3)$, $\text{CH}(\text{OH})\text{CH}_3$, CHO , or H ;



R_6 is CH_3 or H;

R_7 is CH_3 or H;

R_8 is CH_3 , CH_2OH or H

R_9 is CH_3 or H;

Y is OH, CH_3 , or H;

W is Cl or H; and

Z is Cl or H.

(b) at least about a stoichiometric amount of a base capable of forming a pharmaceutically acceptable salt with a lipophilic oligosaccharide antibiotic of Formula I;

(c) an amount of binding agent sufficient to achieve efficacious delivery of said lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding adverse reaction syndrome; and

(d) 0% by weight (basis, total weight in said composition) up to an iso-osmotic amount of a pharmaceutically acceptable tonicity agent.

2. The composition of claim 2 wherein the lipophilic oligosaccharide antibiotics represented by Formula 1 are selected from flambamycin, the everminomicins, the everminomicin-type antibiotics, curamycin, and the avilamycin A-N antibiotics.

3. The composition of claim 1, wherein the base is selected from chloroprocaine, procaine, piperazine, glucamine, N-methylglucamine, N'-N-dimethylglucamine, ethylenediamine, diethanolamine, diisopropylamine, diethylamine, N-benzyl-2-phenylethylamine, N,N'-dibenzylethylenediamine, choline, clemizole, tris(hydroxymethyl)aminomethane, D-glucosamine or sodium hydroxide.

4. The composition of claim 1 wherein the base is N-methylglucamine.

5. The composition of claim 1 wherein the binding agent is HSA.

7.

(a)

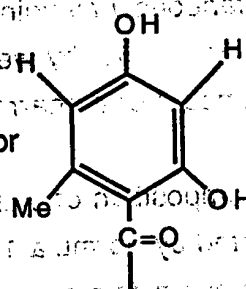


wherein X is one of NO₂, NO, NHOH, NH₂, NHCOCH₃, NHC₂H₅, N(C₂H₅)₂, OH or H

R_2 is H or CH_3

R_4 is H or $CH(OCH_3)(CH_3)$

R₅ is H or



(b)

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8. The composition of claim 7 wherein the base is selected from chloroprocaine, procaine, piperazine, glucamine, N-methylglucamine, N,N'-dimethylglucamine, ethylenediamine, diethanolamine, diisopropylamine, diethylamine, N-benzyl-2-phenylethylamine, N,N'-dibenzylethylenediamine, choline, clemizole, tris(hydroxymethyl)aminomethane, D-glucosamine or sodium hydroxide.

9. The composition of claim 7 wherein the base is N-methylglucamine.

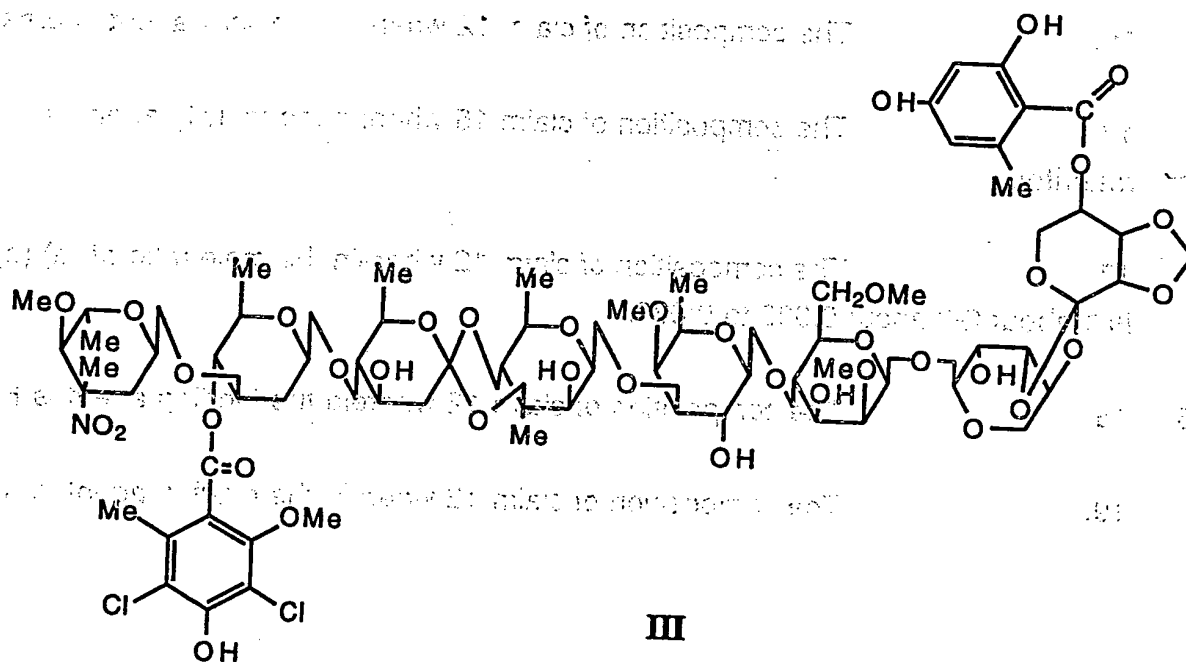
10. The composition of claim 7 wherein the binding agent is HSA.

11. The composition of claim 7 wherein the binding agent is rHA.

12. A composition of matter comprising:

- (a) the lipophilic oligosaccharide antibiotic represented by Formula III

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III

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- (b) at least about two equivalents of a base (per mole of the compound of Formula III) capable of forming a pharmaceutically acceptable salt of

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said lipophilic oligosaccharide antibiotic of Formula III (c) an amount of a binding agent sufficient to achieve efficacious delivery of said lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding occurrence of adverse reaction syndrome; and (d) 0 % by weight (basis, total weight of said composition) of up to an iso-osmotic amount a pharmaceutically acceptable tonicity agent.

13. The composition of claim 8 wherein the base is selected from chlorprocaine, procaine, piperazine, glucamine, N-methylglucamine, N,N-dimethylglucamine, ethylenediamine, diethanolamine, diisopropylamine, 10 diethylamine, N-benzyl-2-phenylethylamine, N,N'-dibenzylethylenediamine, choline, clemizole, tris(hydroxymethyl)aminomethane, D-glucosamine or sodium hydroxide.

14. The composition of claim 12 wherein the base is N-methylglucamine. 15

15. The composition of claim 12 wherein a tonicity agent is present.

16. The composition of claim 15 wherein the tonicity agent is mannitol. 20

17. The composition of claim 12 wherein the mole ratio of (a):(b):(c) is 1:about 2-6:about 0.006 to 0.030. 25

18. The composition of claim 12 wherein the binding agent is HSA.

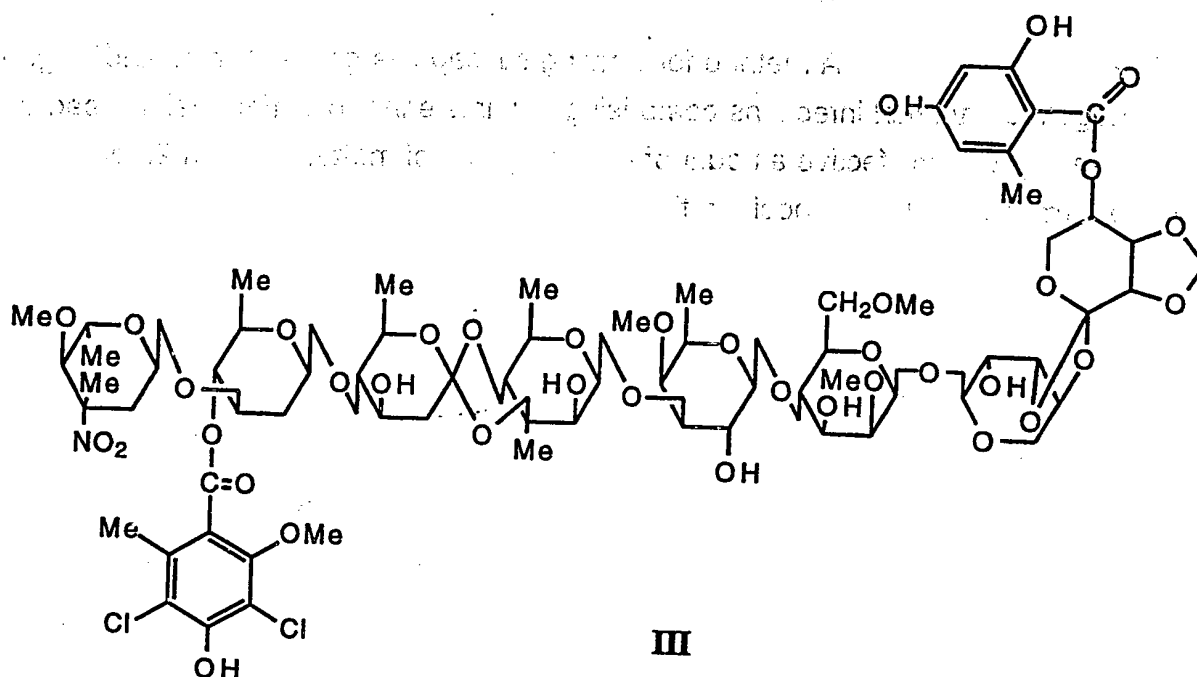
19. The composition of claim 12 wherein the binding agent is rHA.

20.

A composition of matter comprising

(a) the lipophilic oligosaccharide antibiotic represented by
Formula III.

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(b) at least about two equivalents of a base (per mole of said antibiotic of Formula III) capable of forming a pharmaceutically acceptable salt of said antibiotic of Formula III (c) an amount of recombinant human albumin sufficient to achieve efficacious delivery of said lipophilic oligosaccharide antibiotic to the serum of an animal while simultaneously avoiding occurrence of adverse reaction syndrome; and (d) an iso-osmotic amount of mannitol as a tonicity agent.

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21. The composition of claim 20 wherein the base is N-methylglucamine.

22. The composition of claim 20 wherein the iso-osmotic amount of mannitol is 35 to 45 % by weight of total composition.

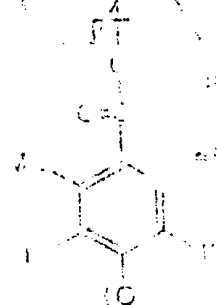
23. The composition of claim 20 wherein the molar ratio of (a):(b):(c) is 1:about 3-3.5: about 0.018 to 0.030.

24. The composition of claim 20 wherein the binding agent is HSA

25. The composition of claim 20 wherein the binding agent is rHA.

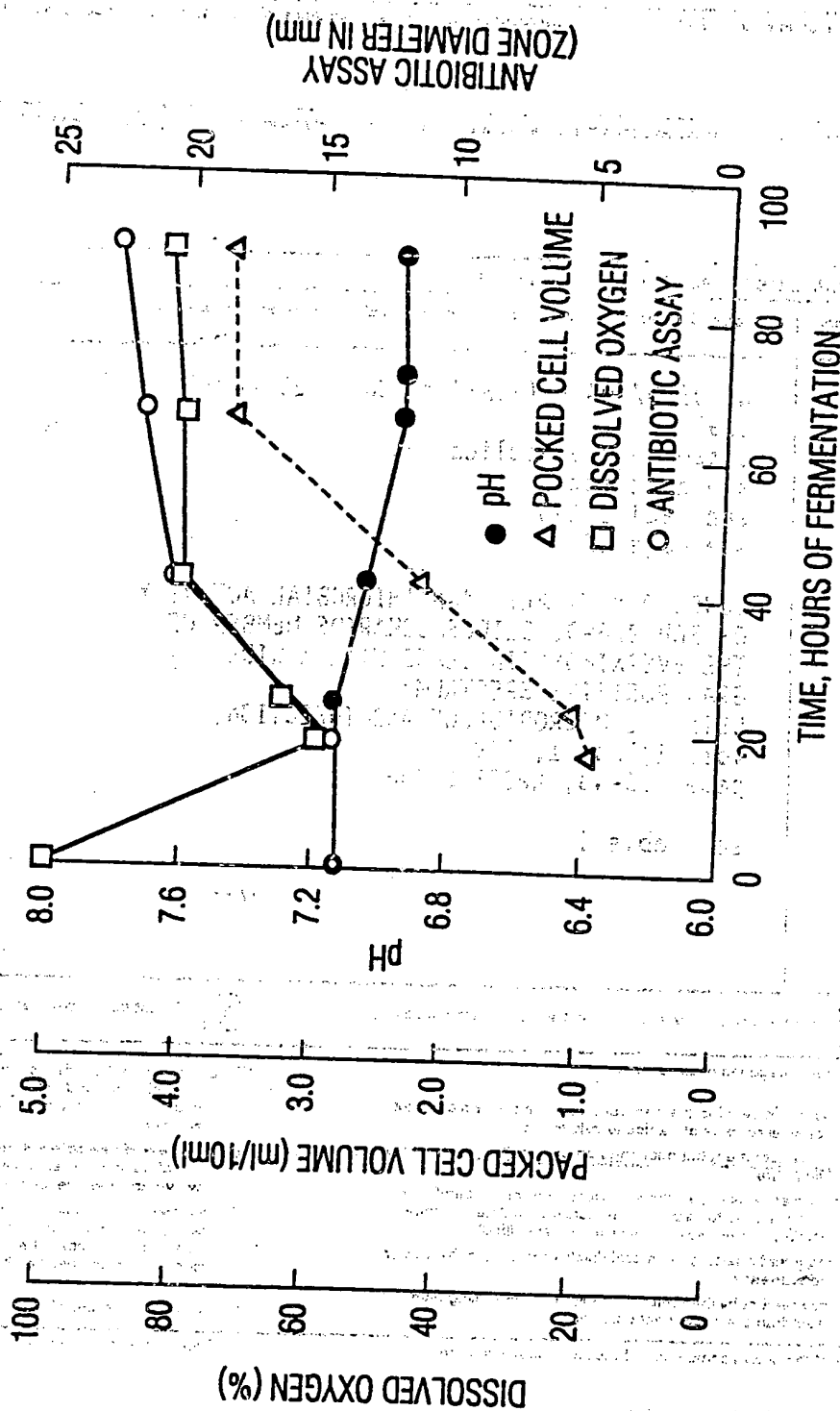
26. A pharmaceutical composition for treating susceptible gram-positive and/or gram-negative bacterial infections comprising an antiinfective amount of a composition of matter of claim 20 and a pharmaceutically acceptable carrier thereof.

27. A method for treating susceptible gram-positive and/or gram-negative bacterial infections comprising administering to a mammal in need of such treating an antiinfective amount of a composition of matter of claim 20 or pharmaceutically composition thereof.



1/1

FIGURE



INTERNATIONAL SEARCH REPORT

 national Application No
 PCT/US 97/22518

 A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 A61K47/48 A61K47/42 A61K38/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

 Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93-07904 A (SCHERING CORP) 29 April 1993 cited in the application	1-4,7-9, 12-17
Y	see abstract see tables 1-3 see page 16	1-27
X	JONES R N ET AL: "ANTIMICROBIAL ACTIVITY OF SCH 27899, OLIGOSACCHARIDE MEMBER OF THE EVERNIMOMYCIN CLASS WITH A WIDE GRAM-POSITIVE SPECTRUM" CLINICAL MICROBIOLOGY AND INFECTION, vol. 1, no. 1, 1995, pages 35-43, XP000652948	1-4,7-9, 12-17
Y	see table 1	1-27

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

22 May 1998

Date of mailing of the international search report

11. 06.98

 Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk

Authorized officer

INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 97/22518

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LORRAIN JM: "Antibiotiques: interaction avec d'autres médicaments" NOUV PRESSE MED, 12 NOV 1979, VOL. 8, NO. 44, PAGE(S) 3653-8, XP002065662 see abstract see page 3654	1-27
Y	GURKAN H ET AL: "Streptomycin sulphate microspheres: dissolution rate studies and release kinetics. I." J MICROENCAPSUL, JAN-MAR 1987, VOL. 4, NO. 1, PAGE(S) 39-46, XP002065663 see abstract see figures 3,5	1-27
Y	NAKASHIO S ET AL: "EVERNINOMICIN, A NEW OLIGOSACCHARIDE ANTIBIOTIC: ITS ANTIMICROBIAL ACTIVITY, POST-ANTIBIOTIC EFFECT AND SYNERGISTIC BACTERICIDAL ACTIVITY" DRUGS UNDER EXPERIMENTAL AND CLINICAL RESEARCH, vol. 21, no. 1, 1995, pages 7-16, XP000653350 see abstract see tables 1,2	1-27
Y	GANGULY A K ET AL: "CHEMICAL MODIFICATION OF EVERNINOMICINS" JOURNAL OF ANTIBIOTICS, vol. 35, no. 5, May 1982, pages 561-570, XP000651703 see page 561	1-27
Y	EP 0 359 148 A (ISHIHARA MINING & CHEMICAL CO ;GREEN CROSS CORP (JP)) 21 March 1990 see abstract see examples	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/22518**Box I** Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 27 is directed to a method of treatment of the human/animal body, a search has been carried out, based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-17 in part
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1-17 in part

In view of the large number of compounds, which are defined by the general definition in claims 1-17, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, Chapter III, paragraph 2.3).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	12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